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Hemipenes eversion behavior: a new form of communication in two *Liolaemus* lizards (Iguania: Liolaemidae)

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Abstract

Males of several animals have intromittent organs and may use these in a communicative context during sexual or intrasexual interactions. In some lizards there have been observations of hemipenes eversion behavior, and the aim of this study is to find out whether this behavior is functionally significant, under a communicative approach. Here, we investigated hemipenes' eversion in two species of *Liolaemus* (*L. coeruleus* Cei and Ortiz-Zapata, 1983 and *L. quilmes* Etheridge, 1993) by filming the response of male focal lizards in different experimental settings: (1) - an agonistic context i.e., with a conspecific male; (2)- a sexual context, i.e., with a conspecific female and (3)- a control treatment, i.e., without a treatment lizard. In both species, focal lizards showed this behavior only in agonistic contexts, with interspecific differences. *L. coeruleus* has longer times until eversion and dragging of hemipenes; however, it has shorter time of eversion and exposition of hemipenes. While *L. quilmes*, has an opposite pattern with respect to *L. coeruleus*. These indicate that hemipenes' eversion can act as a visual display and as a signal of aggressive behavior towards conspecific rival males. The present study offers a new, behavioral perspective on the use of masculine genitalia in lizards.

Key words: Squamata, visual displays, *L. coeruleus*, *L. quilmes*, male genitalia

Introduction

In many groups of animals, males use intromittent organs to transfer their sperm during

copulation (Smith 1984). The evolution of these organs is generally believed to be driven by

selective factors related to sexual selection, ecology and female genitalia morphology

(Langerhans et al. 2016), which result in a high morphological variety (de Souza et al. 2014).

Male intromittent organs can be used as communicative signalers, mainly during courtship, as

occurs in some insects (e.g., flies, beetles, wasps), fish (e.g., guppy) and different placentary

(e.g., monkeys, elephants, cavy, rodents, shrews) and marsupial (e.g., wallabies) mammals

(Eisenberg et al. 1971; LaFollette 1971; West-Eberhard 1984; Stralendorff 1986; Eberhard 1990;

Brooks and Caithness 1995; Maestripieri 2005; Ottway et al. 2005; Briceño et al. 2010).

Furthermore, in mammals, the erect penis may send signals indicating subordination (e.g.,

Rozenfeld and Rasmont 1991; East et al. 1993; Kutsukake and Castles 2004; Liebal et al. 2004)

or dominance (e.g., Ploog and MacLean 1963; LaFollette 1971; Rozenfeld and Rasmont 1991)

among males.

Considering reptiles, males of the Squamata order have notably complex masculine

genitalia, called hemipenes (e.g., Arnold 1986*a*; Böhme and Ziegler 2008; Lee et al. 2015). They

are formed by two eversible, sac-like structures that are frequently covered with different

ornamentations such as calyces, papillae, flounces, and spines (Dowling and Duellman 1978).

Until now, research on lizard hemipenes has focused mainly on their reproductive function (e.g.,

Conner and Crews 1980; Arnold 1986*b*) and their morphological characteristics (e.g., Böhme and

Ziegler 2008; Quipildor et al. 2018). Although behavioral aspects have been given much less

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attention, there are some studies that explore copulation's behavior (e.g., Tokarz 1989; Shine et al. 2000; Moreira and Birkhead 2004). Another interesting behavioral approach was made by in den Bosch (2001), who mentioned that during the breeding season, lacertid lizards shed their genital skin and deposit hemipenial blobs, while everting and dragging their hemipenes.

Liolaemus is a highly diverse genus of South American lizards with more than 260 species (Uetz et al. 2018). It is divided into two monophyletic groups, the Chilean group or *Liolaemus sensu estricto* and the Argentinean group or *Eulaemus* (Laurent 1983). Taking into account the possibility of interspecific differences, we studied two *Liolaemus* species: *L. coeruleus* Cei and Ortiz-Zapata, 1983 belonging to the Chilean group and *L. quilmes* Etheridge, 1993 of the Argentinean group. Ruiz-Monachesi (2018) noted that *Liolaemus coeruleus* and *L. quilmes* males have a particular behavior of hemipenes eversion (similar to in den Bosch 2001), which potentially may be use as a visual display (unpublished data, PhD thesis; Ruiz-Monachesi 2018). This is an uncommon behavior in these lizards, which perform mainly others visual displays such as head-bob movement and forelimbs waves (Halloy 1996, 2012; Halloy and Castillo 2002; Martins et al. 2004; Labra et al. 2007; Vicente and Halloy 2015; Ruiz-Monachesi 2018; Vicente 2018). However, he did not determine whether the social context affects this behavior. Conversely to in den Bosch (2001), Ruiz-Monachesi's (2018) observations were not carried out during the breeding season and lizards did not deposit hemipenial blobs nor did they change their genital skin. Based on Ruiz-Monachesi (2018) observations, we try to answer the question whether this behavior could be a visual communication mechanism and, for the first time, give a detailed description of its function in *Liolaemus* lizards. Additionally, we examined whether the behavior is influenced by different social contexts, such as agonistic (i.e., in presence of a conspecific male) or sexual (i.e., in presence of a conspecific female) encounters. We

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expected to observe hemipenes eversion in sexual contexts, given that hemipenes have a reproductive function.

In *Liolaemus*, the precloacal pores are a source of chemical secretions (Valdecantos et al. 2014) with pheromonal properties (Labra et al. 2005; Valdecantos and Labra 2017). In other lizard genera, it was proposed that the absence of pheromonal pores might be associated to an increased use of visual displays (e.g., Lacertidae and Phrynosomatidae; Hews and Benard 2001; Baeckens et al. 2015). Based on the fact that lizards of the Chilean group have less precloacal pores than those of the Argentinean group (Laurent 1983; Jara et al. 2018), Martins et al. (2004) hypothesized that the former depended more on visual communication, while the latter might use the chemical modality more. Males of *L. quilmes* have an average of 5.8 precloacal pores (Etheridge 1993), but *L. coeruleus* males lack these pores completely (Cei and Ortiz-Zapata 1983). Considering the above-mentioned, we expect *L. coeruleus* to rely more heavily on visual displays than *L. quilmes*, therefore exposing their hemipenes longer. Additionally, as the number of tongue flicks is considered as a proxy to chemical exploration (Font and Desfilis 2002; Baeckens et al. 2017*a*) we expect *L. coeruleus* to make fewer tongue flicks than *L. quilmes*.

Materials and methods

In November 2015, we collected 20 adults of *Liolaemus coeruleus* (10 males and 10 females) near Alumine, Neuquén (Route 13 between Kilka and Primeros Pinos: 38° 54' 14.70"S; 70° 43' 59.50"W; datum WGS84). Additionally, 15 specimens of *L. quilmes* (9 males and 6 females) were collected near to Rio Seco, Cafayate, Salta (26º07'22.9" S; 65º58'06.1"W; datum WGS84), in November 2016. Both species were sampled during the post-hibernation season. Lizards were captured by hand (*L. coeruleus*) or using a loop (*L. quilmes*) and kept in individual cloth bags until their arrival at the laboratory. Then, they were placed in individual plastic

enclosures (36 × 27 ×19 cm) covered with a lid of plastic mesh. Enclosures contained 3 cm of sandy substrate, a rock to be used as shelter and basking place, and a small bowl with water *ad libitum*. Lizards were kept in an isolated room with a summer photoperiod of 13:11, *L*: *D*, using halogen lamps, which maintained a mean ambient temperature of 30 $°C \pm 2 °C$ during the light phase. Every other day we fed each lizard with two *Tenebrio mollitor* larvae, dusted with vitamins. Prior to the experiments, lizards remained undisturbed in their enclosures for one week, allowing them to get used to the experimental conditions.

Appropriate actions were taken to minimize the stress of lizards. The study was conducted in accordance with international standards on animal welfare and is compliant with national regulations and the "Comité Nacional de Ética en la Ciencia y la Tecnología" of Argentina (Expte. 5344/99 Res. 1047). At the end of all experiments, lizards were sacrificed with a pericardic Pentothal injection, following the standard protocol (Scrocchi and Kretzschmar 1996). Lizards were fixed in formol 10 % and conserved in ethanol 70%, for their use in systematic studies and their final deposition in the herpetological collection of the Instituto de Bio y Geociencias del NOA (IBIGEO).These procedures are approved by the ethical use of animals of IBIGEO and take into account animal welfare regulations. Animals were collected with the permits N° 4351-0026/2014 (*L. coeruleus*) and Nº 815/13 (*L. quilmes*).

Experimental design

We filmed the behavioral response of a male focal lizard placed in sight of a treatment lizard of similar size, i.e., with a minimal snout-vent length difference between both lizards (focal and treatment; maximum difference 2.57 mm; Table 1 and 2), avoiding a possible effect of body size (e.g., Labra 2006). Each lizard performed three trials: (1) - in an agonistic context, with a

male individual; (2) - in a sexual context, with a conspecific female; (3) -in a control treatment, without a treatment lizard. We used a glass enclosure for the experiments, equipped with clean (i.e., without chemical scents) sandy substrate and divided by a transparent glass sheet into two equally sized sectors of 30 cm in length (Fig. 1). Before each trial, focal and treatment lizards were removed from their enclosures and held in an individual cloth bag for 10 min, to minimize handling stress (e.g., Labra 2011). The experiment started with the introduction of the treatment lizard into one side of the experimental glass enclosure (60 cm $L \times 20$ cm $W \times 30$ cm H). Then, the cloth bag with the focal lizard was opened to allow the animal to move freely into the opposite sector of the glass enclosure (30 cm L). Once the focal lizard entered the glass enclosure and we were out of its visual field, we registered the time of latency to the first movement with a digital stopwatch. Latency time can be defined as the time that passes from the moment the lizard enters the glass enclosure until it sees the treatment lizard and begins to move. After latency time, we videotaped the behavior of the focal lizard for 10 min; using two digital video-cameras, Sony DCR-SR67 and JVC GZ-EX210BU, installed at 40 cm and 20 cm in frontal and lateral views, respectively (Fig. 1). We saved the digital videos for further analyses, which were performed with VLC Media Player 2.2.1. All focal lizards responded with certain latency, but if latency time exceeded seven minutes, the trial was canceled and repeated on a different day. After every trial, we assured that focal and treatment lizards were healthy. Then, lizards were returned to their enclosures, and remained undisturbed for at least three days before a new trial. To avoid cross-contamination, we changed gloves, cleaned glass enclosures with alcohol 96%, and discarded the experimental substrate after every trial. Each one of these trials was randomized until each individual completed all trials.

From videos, we recorded the behaviors mentioned in Tables (1and 2) and we analyzed the following variables (*s*) only in those videos where the hemipenes eversion behavior was present:

(1) Time of the first defecation: time elapsed between the first movement to the lizard's first defecation.

(2) Time until the eversion: time between the lizard's first defecation and eversion of the hemipenes.

(3) Duration of the eversion: total time in which hemipenes are everted but not dragged.

(4) Duration of dragging: total time in which hemipenes and cloaca are dragged through the substrate.

(5) Number of tongue flicks: as an index of chemical exploration (Font and Desfilis, 2002) that considers the times that the lizard protrudes and rapidly retracts its tongue, regardless of whether the tongue touches the substrate, wall or if it is waved in the air (e.g., Labra 2006).

Statistical analyses

Because our first objective was to test differences between treatments, and not between species, first we analyzed data of both species jointly (*n* =19). From videos analyzes, we observed that those animals that everted their hemipenes only did this once. For this reason we used a Cochran *Q* test, considering that our response variable only takes two possible outcomes (0 = absence of this behavior; 1 = presence of behavior). On the other hand, interspecific differences (*Liolaemus coeruleus* vs. *L. quilmes*) were analyzed only considering those individuals that presented this behavior (*n* = 7). As neither our response variable nor the transformed fulfilled assumptions of normality and homoscedasticity, we used a Mann-Whitney

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U test, with species as grouping factor. Data analysis was performed using STATISTICA Software, version 7.0.

To analyze the number of tongue flicks (*n* =19) as a response variable, we used the following predictor variables: "Species" (*Liolaemus coeruleus*, *L. quilmes*), "Condition" (focal, treatment) and "Hemipenes Behavior" (presence, absence). We performed generalized linear mixed models (GLMMS) using R (R Core Team, 2015). The species, condition and hemipenes behavior were fixed effects, whereas the individual identity of the subject lizard was considered as a random effect. Because our data are over dispersed (Zuur et al. 2009), our models had binomial negative distribution. Because of this, we implemented a binomial negative distribution with log-link function, using the glmmADMB package (Skaug et al. 2014). We explored all possible models and chose the best-fitted model following the Akaike information criterion (Burnham and Anderson 2004). Analyses were followed by post-hoc Fisher *LSD* tests performed with the agricolae package (Mendiburu 2015).

Results

Description of hemipenes eversion behavior

After the first visual contact, hemipenes eversion behavior can be divided into five phases. These phases were always performed in the same manner by all individuals, regardless of their species: I- The lizard defecates, moves and remains immobile with its body, head and limbs (anterior and posterior) pressed against the substrate (Fig. 2 A). II-The lizard elevates torso and head (movement 1, Fig. 2 B), doing a semi-flexion of anterior limbs, holding this position for one second (approximately). III- Posterior limbs are lifted and semi-flexed forwards (movement 1, Fig. 2 C); while the anterior limbs are moved towards the posterior extreme (movement 2, Fig. 2

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C). IV- Head and anterior limbs move rapidly downwards (movement 1, Fig. 2 D). Posterior

limbs and back are lifted pointing backwards (movement 2, Fig. 2 D) and hemipenes are everted.

V- Back and cloaca move downwards (movement 1, Fig. 2 E); then the lizard advances, dragging

its hemipenes and cloaca on the sandy substrate (movement 2, Fig. 2 E).

Social context experiments

Overall, seven focal lizards everted their hemipenes. Four (40%) of ten males of

Liolaemus coeruleus (Table 1) and three of nine (33.33 %) *L. quilmes* (Table 2), displayed this

behavior. Moreover, the full sequence was visual contact, defecation, hemipenes eversion and

dragging (see supplementary data S1, S2). The treatment analysis showed significant differences

among them (Cochran Q test, $Q_{[df=2, n=19]}$ = 14; P = 0.0032). The behavior was only observed in

the agonistic treatment in seven individuals of both species (*L. coeruleus* = 4; *L. quilmes* = 3).

Neither the control nor the sexual context triggered hemipenes eversion. The Mann-Whitney U

test (see Table 3) in these seven individuals, revealed species-specific differences in all variables

except for the time of first defecation (Table 3, Fig. 3). Time until eversion was longer in *L.*

coeruleus than in *L. quilmes* (Fig. 3 A); conversely the latter showed a longer duration of the

eversion than *L. coeruleus* (Table 3; Fig. 3 B). Finally, the duration of dragging was longer in *L.*

coeruleus than in *L. quilmes* (Fig. 3 C).

With regard to the number of tongue flicks (TF) made in agonistic context and its

relationship with the factors: "Species" (SP), "Condition" (COND) and "Hemipenes Behavior"

(HB); in total 11 models were generated (Table 4). Following Akaike's criterion, only one model

was most informative: TF~ COND*SP (Table 4). This model also was statically significant (P =

0.001). The factors COND and SP, were not statically significant (F_{COND} = 0.04, P_{COND}= 0.86; F

$_{COND}$ = 0.19, P_{SP} = 0.16); however COND*SP did have statically significant differences (F= 7.23,

P = 0.001). Posthoc analyses did show that *Liolaemus coeruleus* focal lizards differ from *L. quilmes* focal lizards (Z= 2.9 P=0.017), while they did not differ from the treatment lizards ($Z_{L. coeruleus}$ = 0.9 $P_{L. coeruleus}$ = 0.25; $Z_{L. quilmes}$ = 0.4 $P_{L. quilmes}$ =0.54). Under focal condition, *L. coeruleus* made less tongue flicks than *L. quilmes* ($\overline{X}_{L. coeruleus}$ = 3.10 ± $SE_{L. coeruleus}$ = 2.07; $\overline{X}_{L. quilmes}$ =11.78 ± $SE_{L. quilmes}$ = 2.20; Fig. 3D).

Discussion

The male intromittent organs in several animals have a sexual function, which have been mainly explored from a copulatory perspective (Smith 1984; Langerhans et al. 2016). However, there are numerous examples in nature which indicate their use in intersexual communicative contexts (e.g., West-Eberhard 1984; Maestripieri 2005; Briceño et al. 2010) and/or intrasexual (e.g., Ploog and MacLean 1963; LaFollette 1971; Rozenfeld and Rasmont 1991). In Squamates, there are no studies which tackle the communicative function of hemipenes. However Bohme (1983) suggested that the lizard *Plica plica* (Linnaeus, 1758), might use its hemipenes as a visual signaler during courtship, but there are no previous mentions of possible agonistic communicative function for hemipenes in Squamata. Here, we described the hemipenes eversion behavior in *Liolaemus* lizards in absence of a sexual context (Fig. 2). Our experiments suggest that the presence of a conspecific male can induce this behavior (S1, S2). Therefore, we can hypothesize that it is used as an aggressive display among males in *Liolaemus* lizards. Additionally, we observed differences between both species; *L. coeruleus* and *L. quilmes* (Fig. 3). We suppose that this behavior has evolved under a complex communication context.

Contrary to our expectations, hemipenes eversion did not occur in sexual situations, i.e., the presence of a female did not trigger males to display their hemipenes. This is surprising but

could be explained by the fact that we conducted the study shortly after the hibernation season and lizards might prioritize other necessities, e.g., foraging activities and territorial defense, over reproduction.

Similar to the previously mentioned observations of hemipenes eversions in lacertids lizards (in den Bosch 2001), we saw that *Liolaemus* lizards defecated, everted their hemipenes and dragged them on the substrate. However, we did not observe the deposition of hemipenial blobs with viscous fluids, nor did we find any skin rests as they were described by in den Bosch (2001). It is possible that the dragging of hemipenes might serve to leave chemical signals and scents related to territorial marking, associated with a space defense behavior (Alberts 1992), since the presence of proctodeal glands (Burkholder and Tanner 1974; Valdecantos et al. 2015) possibly implies there is chemical secretion during this behavior as we will discuss below.

As hemipenes eversion only occurred in agonistic contexts, always after defecation of the focal lizard and frequently followed by an appeasing behavior of the receptor lizard, such as closing eyes ,tongue-flicking or tail waving (see Fox and Shipman 2003; S2), we suppose that it is a form of aggressive behavior between two males. Here, we also observed other visuals displays in both species, such as head-bobs, charges, forelimb waves, accompanying the hemipenes eversion behavior (see Tables 1, 2 and S2). In concordance with our proposal, these behaviors are known to be used as signs of aggressiveness in other lizards (e.g., Ord 2001; Wilczynski et al. 2015; Peters et al. 2016). Interestingly, similar behaviors have been registered in some mammals, which also drag their penis and their anogenital region (Rozenfeld and Rasmont 1991; Ottway et al. 2005) during agonistic interactions. For example the males of bank vole drag their penis indicating a dominance through chemical and visual signals (Rozenfeld and Rasmont 1991). Possibly in mammals, this behavior has a double communicative function: visual

and chemical, since the prepuce in some mammals present chemical glands (e.g., Clapperton et al. 1987). Furthermore, males of bank vole and others mammals such as elephants, defecate prior to the exposition of the penis (Eisenberg et al. 1971; Rozenfeld and Rasmont 1991) as we observed in both *Liolaemus* species. This may indicate that feces are an important component of this agonistic behavior in different taxa.

We found several significant differences between *Liolaemus coeruleus* and *L. quilmes*, as the former lacks precloacal pores (Cei and Ortiz-Zapata 1983), which was thought to be an indication of a lower ability to use scents (Hews and Benard 2001). Therefore, we assumed that *L. coeruleus* would invest more in visual signals than *L. quilmes*. Contrary to this assumption, *L. coeruleus* males invested less time in the visual display of their hemipenes than *L. quilmes* males (Fig. 3 B). Furthermore, *L. coeruleus* had a longer dragging phase than *L. quilmes* (Fig. 3 C). On another hand, in concordance with our initial assumption, *L. coeruleus* showed a lower number of tongue flicks than *L. quilmes* (Fig. 3D). This is interesting since tongue flicks can be considered as a proxy to chemical exploration (Baeckens et al. 2017*a*), thus *L. coeruleus* may present a greater investment in chemical signaling (dragging), but a smaller investment in chemical exploration (tongue flicks). Despite the fact that *L. coeruleus* males lack precloacal pores, they bear proctodeal glands (Valdecantos et al. 2015), similar to others lizards (e.g., *Sceloporus graciosus* Baird and Girard, 1852; Burkholder and Tanner 1974) and possibly uses them during the process of hemipenial dragging. In this case, a longer duration of dragging may help to increase chemical scents. On the other hand, *L. quilmes*, a species with precloacal pores (Etheridge 1993), which facilitate chemo-depositions (Baeckens et al. 2017*b*), everts its hemipenes more quickly (Fig. 2 A) and spends more time showing them (Fig. 3 B) than *L. coeruleus*. However, it presents a heavier investment in chemical exploration (Fig. 3 D), which

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indicates that *L. quilmes* may use hemipenes eversion as a visual rather than a chemical signal (Fig. 3 C). Thus, *L. quilmes* may present a smaller investment in chemical signaling (dragging), but a greater investment in chemical exploration (tongue flicks). One possibility is that males use this behavior to counterbalance their chemical morphological shortcomings (more or less chemical glands and tongue flicks). Future researches are needed to test whether the hemipenes dragging behavior leaves a chemical trail by analyzing the response of both lizards (tongue-flicks), to stimulus offered on a cotton-tip with hemipenes scents and/or to observe lizards behavior in an arena with these scents. Another possibility is that these interspecific differences are a consequence of phylogenetic effect, since both lizards represent the two main clades in *Liolaemus* (Laurent 1983). *L. coeruleus* belongs to the Chilean group (Cei and Ortiz-Zapata 1983) and *L. quilmes* belongs to the Argentinean group (Etheridge 1993). Future research with a more extensive taxon sampling is needed to test whether this behavior is present in different groups of both clades and whether they present significant differences. Regardless, it must be noted that our results showed that this behavior is present in the two main clades of *Liolaemus*, which may suggest that it is presents in other species of this genus.

If we consider the possibility of a double communicative function of this behavior, then it must be analyzed in the context of multimodal communication (Bakker and Traniello 2013). Multimodal communication implies that two or more different sensory modalities act together to send and receive information during communication (Bro-Jørgensen 2010). The hemipenes eversion behavior in *Liolaemus* may involve two sensorial modalities, a chemical and a visual one. The eversion of hemipenes, as well as the feces may act as visual signals, while feces and hemipenial secretions may serve as chemical signals (in den Bosch 2001; Labra et al. 2002). Some studies in *Liolaemus* lizards suggest that chemical and visual modalities are positively

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associated (Thompson 2002; Martins et al. 2004; Labra et al. 2007; Vicente and Halloy 2017).

Hence, if an individual is able to use both sensory modalities of the eversion behavior, its

message would be reinforced (Partan and Marler 2005).

In summary, we registered and described for the first time the hemipenes eversion

behavior in *Liolaemus* lizards. Our experiments showed that the presence of a conspecific male

can induce hemipenes eversion. We hypothesize that this behavior may be an aggressive display

among males in *Liolaemus* lizards. On the other hand, we observed that *L. coeruleus* spent more

time in the dragging phase (potential scent marking) and made fewer tongue flicks (less chemical

exploration); while *L. quilmes* spent more time showing its hemipenes (visual signaling) and

made more tongue flicks (less chemical exploration). In both species, this behavior seems to

counterbalance their main form of communication, i.e., the presence of precloacal pores

accompanies a potentially more visual signaling, while the opposite is true for the absence of

precloacal pores. We consider this as a possible complex communication and hypothesize that the

hemipenes eversion behavior could be used as a chemical and visual (multimodal)

communication with a territorial function. The present study may be a starting point for the study

of masculine genitalia in lizards outside the systematic or sexual context considering a

communicative approach. Finally as we had a low sample size, a more thorough research, which

increases it, is necessary to have a better idea of how general and common this particular

behavior is in lizards.

Acknowledgments

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Supplementary data

S1 video: *Liolaemus coeruleus* showing hemipenes eversion behavior.

S2 video: *Liolaemus quilmes* showing hemipenes eversion behavior.

Figure captions

Figure 1: Drawing representing the experimental design used to film the behavioral response of lizards, showing the glass enclosure divided by a transparent glass sheet, the focal (black) and treatment (grey) lizards and two digital video-cameras installed at 40 cm and 20 cm in frontal and lateral views, respectively.

Figure 2: Drawing (left) and filmed sequence (right) showing the hemipenes eversion behavior in *Liolaemus*: A- defecation and beginning of the behavior; B- lifting of anterior extreme; C- lifting of posterior extreme; D- hemipenes eversion; E- dragging. Internal numbers indicate the sequence order; black arrows show the direction of movements. Right and left, represent anterior and posterior extremes, respectively. The black circle in B signals feces.

Figure 3: Main differences between *Liolaemus coeruleus* (white) and *L. quilmes* (black) observed in agonistic context. Columns represent mean values, bar lines represent + the standard error of A- time until eversion; B- duration of eversion; C- duration of dragging and D- number of tongue flicks. Asterisk indicates statistically significant differences ($P<0.05$). n =sample size.

Table 1: Summary of results of conspecific male treatment to *Liolaemus coeruleus* showing the presence (*P*) or absence (*A*) of hemipenes eversion behavior (HEB; *n*=10),defecation, i.e., the number of time that a lizard defecates; the number of tongue-flicks made by focal (TF_{Focal}) and treatment ($TF_{Treatment}$) lizards; main visual displays made by focal (Displays $_{Focal}$) and treatment (Displays $_{Treatment}$) lizards and snout-vent length of focal (SVL $_{Focal}$) and treatment lizards (SVL $_{Treatment}$).

HEB	Defecation	TF_{Focal}	TF $_{Treatment}$	Displays $_{Focal}$	Displays $_{Treatment}$	SVL $_{Focal}$	SVL $_{Treatment}$
P	1	1	41	Head-bob; Forelimbs	Closing eye	56.91	58.47
P	1	5	14	Head-bob; Forelimbs	Closing eye	58.47	59.65
P	1	5	0	Head-bob; Forelimbs; Push-up	Closing eye	59.65	60.7
A	0	4	6	Forelimbs	Head-bob; Forelimbs; Moves	60.7	60.85
P	1	6	18	Head-bob; Forelimbs	Closing eye; Forelimb; Escape	60.85	60.99
A	2	4	1	Head-bob; Forelimbs	Head-bob; Forelimbs; Moves	60.99	62.81
A	0	1	3	Head-bob; Forelimbs	Forelimbs; Charges; Closing eye	62.81	62.91
A	1	3	2	Push-up; Forelimbs	Head-bob; Forelimbs; Moves	62.91	63.49
A	1	2	0	Head-bob; Forelimbs	Closing eye; Escape	63.49	66.06
A	0	0	4	Head-bob; Forelimbs; Push-up	Head-bob; Forelimbs; Moves	66.06	63.49

Table 2: Summary of results in conspecific male treatment to *Liolaemus quilmes* showing the presence (*P*) or absence (*A*) of hemipenes eversion behavior (HEB; n=9), the number of time that a lizard defecates; the number of tongue-flicks made by focal (TF_{Focal}) and treatment ($TF_{Treatment}$) lizards; main visual displays made by focal (Displays $_{Focal}$) and treatment (Displays $_{Treatment}$) lizards and snout-vent length of focal (SVL $_{Focal}$) and treatment lizards (SVL $_{Treatment}$).

HEB	Defecation	TF_{Focal}	$TF_{Treatment}$	Displays $_{Focal}$	Displays $_{Treatment}$	SVL Focal	SVL Treatment
P	1	24	0	Head-bob; Push-up; Forelimbs; Moves ; Mouth	Inmovility; Tail waiving	49.6	50.33
A	0	2	4	Head-bob; Forelimbs	Head-bob; Forelimbs; Moves	50.33	50.63
A	2	11	3	Head-bob; Forelimbs; Moves	Head-bob; Moves ; Tail waving	50.63	51.02
A	0	3	7	Head-bob; Push-up; Moves	Forelimbs; Moves	51.02	51.7
A	1	9	7	Moves	Head-bob	51.7	51.02
P	2	4	2	Moves	Moves	53	53.6
A	1	19	2	Push-up	Closing eye	53.6	53
A	0	7	8	Push-up; Forelimb	Head-bob; Push-up; Forelimb	55.83	56.47
P	1	27	2	Head-bob; Moves	Closing eye; Moves	56.47	55.83

Table 3: Results of Mann-Whitney U test showing values of Z (P) and values of mean $\pm$ standard error for conspecific male treatment for males that presented hemipenes eversion behavior was observed (*Liolaemus coeruleus* =4; *L. quilmes* = 3). Response variables different times (s): time of the first defecation; time until eversion; duration of eversion and duration of dragging. Grouping variable: Species (*L. coeruleus*, *L. quilmes*). Statistically significant results (P<0.05) in bold.

Variable	Species	*L. coeruleus* (n=4)	*L. quilmes* (n=3)
Time of the first defecation (s)	-0.35 (0.72)	402 $\pm$ 77.5	406 $\pm$ 89
Time until eversion (s)	**2.12 (0.033)**	14.25 $\pm$ 5.72	1.66 $\pm$ 6.6
Duration of the eversion (s)	**1.18 (0.025)**	4.25 $\pm$ 1.29	12.33 $\pm$ 1.5
Duration of dragging (s)	**2.21< (0.001)**	7.00 $\pm$ 0.83	1.00 $\pm$ 0.96

Table 4: Eleven possible generalized linear mixed models (GLMMS) generated when testing the relation between the number of tongue flicks (TF) and the factors: COND = condition (focal, treatment); HB = hemipenes behavior (present, absent) and SP = species (*Liolaemus coeruleus n*= 10, *L. quilmes n*= 9) for conspecific male treatment. *N°*= number of model; Model= type of model; LogLik= Log-likelihood; AICC= Akaike's value; *Wi* = Akaike's height. In bold the best model in base of Akaike's criterion, i.e., the model with a highest Akaike's weigh.

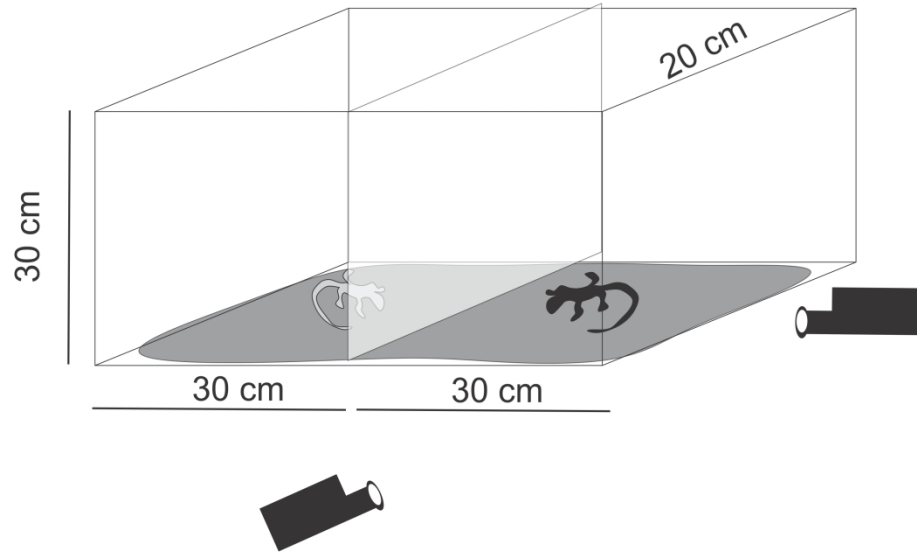| *N°*	Model	LogLik	AICC	*Wi*
1	TF~ COND	-114.65	237.30	0.044
2	TF~ HB	-114.00	235.99	0.086
3	TF~ SP	-113.89	235.77	0.096
4	TF~ COND+HB	-113.83	237.65	0.037
5	TF~ COND+SP	-113.85	237.70	0.036
6	TF~ HB+SP	-113.42	236.83	0.056
7	TF~ COND+ HB+ SP	-112.98	237.96	0.032
8	TF ~ COND*HB	-113.83	237.65	0.037
9	**TF~ COND*SP**	**-110.59**	**233.17**	**0.352**
10	TF ~ HB*SP	-112.17	236.34	0.072
11	TF ~ COND*HB*SP	-109.45	234.90	0.149

Figure 1: Drawing representing the experimental design used to film the behavioral response of lizards, showing the glass enclosure divided by a transparent glass sheet, the focal (black) and treatment (grey) lizards and two digital video-cameras installed at 40 cm and 20 cm in frontal and lateral views, respectively.
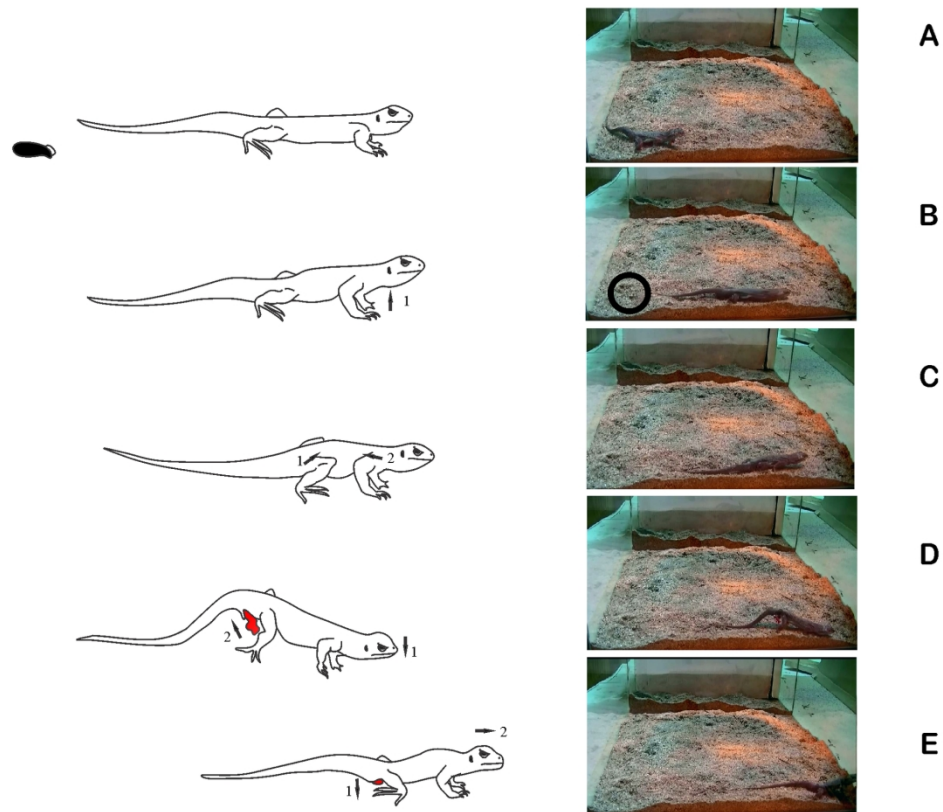
Figure 2: Drawing (left) and filmed sequence (right) showing the hemipenes eversion behavior in *Liolaemus*: A- defecation and beginning of the behavior; B- lifting of anterior extreme; C- lifting of posterior extreme; D- hemipenes eversion; E- dragging. Internal numbers indicate the sequence order; black arrows show the direction of movements. Right and left, represent anterior and posterior extremes, respectively. The black circle in B signals feces.
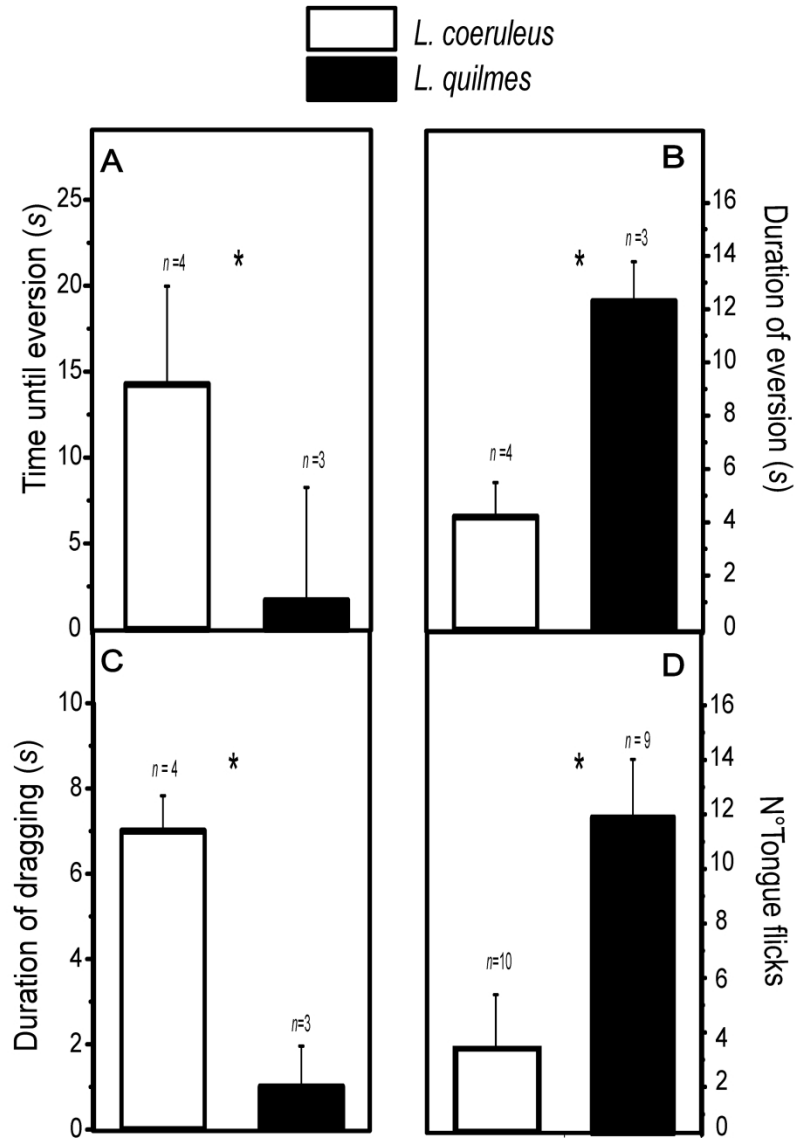
179x156mm (300 x 300 DPI)

Figure 3: Main differences between *Liolaemus coeruleus* (white) and *L. quilmes* (black) observed in agonistic context. Columns represent mean values, bar lines represent + the standard error of A- time until eversion; B- duration of eversion; C- duration of dragging and D- number of tongue flicks. Asterisk indicates statistically significant differences (*P*<0.05). *n* =sample size.

315x430mm (300 x 300 DPI)